Albumin-binding of molecular imaging proteins for biodistribution modification

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Introduction

Rapid tumor imaging is essential in the early detection, patient classification, and determining treatment effectiveness for tumors. One aim of the Hackel Lab is to identify an optimal balance between rapid clearance from background tissue and the extent of tumor targeting. Albumin is a protein with a relatively long half-life compared to other proteins within the body, leading to the hypothesis that if a protein binds to the albumin it will stay in the body for a slightly longer, yet still brief, period of time before being cleared from the bloodstream, which would allow for better imaging.

- The EGFR target-binding protein clone D fibronectin with albumin-binding peptides (DABP, or ABP) identified though DNA sequencing as candidate of interest for albumin-binding experimentation.
- Overall goal: produce 0.5 -1.0 mg of ABP clones for *in vivo* trials on mice.

Production Data

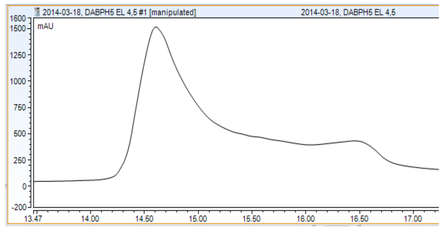

Figure 1 *Representative HPLC Plot:*
High pressure liquid chromatography plot for a sample of DABPH, shown in Arbitrary Units vs. time (min). The area under the curve can be related mathematically to the amount of protein present in a sample; larger area indicates more protein. HPLC area under the curve calculations along with A280 measurements provided the greatest insight to the amount of protein present in a purified sample.

Fluorophore Conjugation

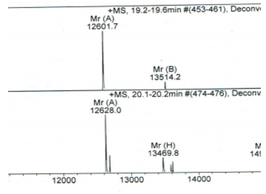

Figure 2 *Mass Spectrometry Data Post-Fluorophore Conjugation:*
A mass spectrometry print-out of a reading done on DABPH post-attempted fluorophore (Dylight-800) conjugation. The numbers shown on the x-axis, along with those above the peaks are molecular weights (g/mol). The above reading shows that the fluorophore may not have completely conjugated; DABPH has a molecular weight of 12605 g/mol (first peak), and a large peak at a molecular weight of approximately 13655 g/mol is expected if the Dylight-800 conjugated to the protein.

Conclusions

Production data for the clones DABPH, X, and L in the form of post-HPLC A280 measurements, HPCL area under the curve calculations, and post-lyophilization A280 measurements were analyzed. After preliminary productions of the clones, the high affinity clone had produced enough to move on to the next stage of experimentation- fluorophore conjugation- while low and no affinity clones produced poorly.

The fluorophore Dylight-800 was attempted to be conjugated to the collected sample of DABPH. To determine if the conjugation was successful the sample was analyzed via mass spectrometer. Complete fluorophore conjugation was not evident based on molecular weight analysis. The next step would have been to add more fluorophore and repeat the process until conjugation was ensured, and the protein would be prepped for use in *in vivo* mice trials. This course of action was put on hold until low and no affinity binders could definitively be produced for controls.

To increase protein production yields DABPH production characteristics were varied. An induction time of 0.5 hours at 37 degrees Celsius was determined to be optimal, and yet production yields of DABPX and L were low. Small scale productions of these clones were performed to determine if this would vary the amount produced, and HPLC area under the curve values increased. This introduced the question of whether purification via spin or gravity column had better protein yields. Initially a larger area under the curve for HPLC plots of spin-purified proteins lent to the conclusion that spin purification would yield much higher amounts, but upon SDS-Page analysis it was shown that the readings were a false positive caused by a contaminant (affibody protein). Spin and gravity columns were concluded to purify with approximately equivalent effectiveness.

Based on the inability to produce high yields of low- and no-binding affinity to albumin clones, the decision was made to halt work on the experiment. DABP production ceased, and DABPH fluorophore conjugation was not revisited in the mindset to save time and materials from being wasted on an objective that is essentially unattainable through the proposed mechanism.

Materials and methods

Three variations of DABP were compared: high affinity (H), low affinity (L), and no affinity (X) for albumin binding. Proteins were created through DNA encoding the proteins of interest that were transformed in to E. coli. Cells were cultured in lysogeny broth (LB) to extract the desired protein at large (1 L) and small-scale (100 mL) levels. The cultures were induced for a varying amount of time and at different temperatures to determine the optimal conditions for ABP protein production.

Production lysate was purified using the protein's characteristic tag of six histidines (His_6) to bind to nickel spin or cobalt gravity columns . Washes and elutions were completed with imidazole at varying concentrations to isolate only ABP.

Measurements of A280 predicted the amount of ABP from the production. High A280 measurement elutions were further purified with high-pressure liquid chromatography(HPLC). Purified protein identity was confirmed through SDS-PAGE and mass spectrometer (MALDI) data. Correctly verified ABP proteins were lyophilized and resuspended in DMSO to be conjugated with near infrared fluorophore for *in vivo* detection.

Purification Comparison

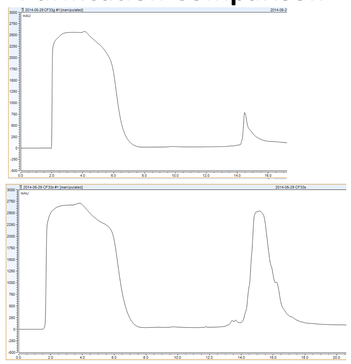

Figure 3 *Purification Comparison HPLC Plots (left):*
High pressure liquid chromatography plots for a purified protein, split into two samples- one sample purified via spin column (Bottom) and the other via drip column (Top). The initial large peak is a solvent shift, and has no importance and no effect on the data.

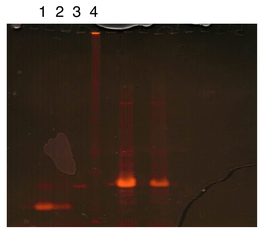

Figure 4 *SDS-Page Verification of Comparison Protein Purity*
SDS-Page uses electrophoresis to separate DNA based on weight. Lanes 1 and 2 are spin column samples, Lane 3 is a gravity column sample, Lane 4 is the ladder for comparison; Lanes that are unlabeled are unrelated proteins. The bright lines at the bottom of lanes 1 and 2 indicate contaminant present.

Literature cited

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Hackel Laboratory

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